

Confocal Imaging of Insulin Stimulated Trafficking of GFP Labeled Epithelial Sodium Channels

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In salt-absorbing epithelial monolayers, the epithelial Na⁺ channel (ENaC) is the rate limiting step for Na⁺ transport. Hormonal regulation of this channel in the principal cells of the distal nephron is important for maintaining salt and fluid homeostasis. Therefore, a complete elucidation of hormone-stimulated signal transduction pathways is important for understanding common aberrations in homeostatic control such as the development of essential hypertension.

Fluorescent protein chimeras of intracellular proteins of interest are valuable tools for investigating the dynamics of cellular mechanisms. In this study stably transfected α ENaC-EGFP clonal lines derived from the A6, renal epithelial cell, parental cell line were used to study the physical mechanisms of insulin-stimulated Na⁺ transport. Within 1 minute of insulin stimulation, ENaC migrates from a diffuse cytoplasmic localization to the apical and lateral membranes. Other studies will be shown that demonstrate after insulin stimulation, phosphatidylinositol 3-kinase (PI3-kinase) is co-localized with ENaC on the lateral but not apical membrane.

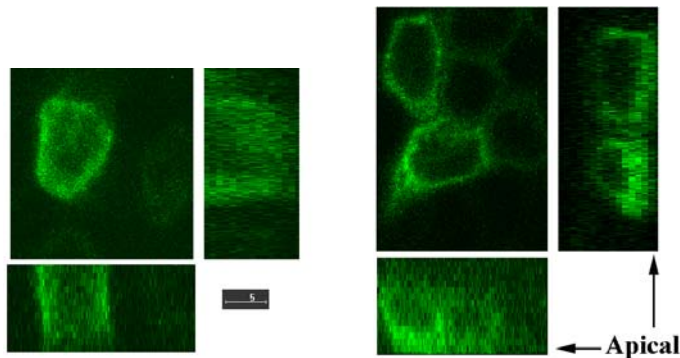


Figure 1: No Insulin

Figure2: 100 nm Insulin

References

- Blazer-Yost, BL, Butterworth M, Hartman Ad, Parker GE, Galetti CJ, Els WJ, and Rhodes SJ.** Characterization and imaging of A6 epithelial cell clones expressing fluorescently labeled EnaC subunits. *Am J Physiol Cell Physiol* 281:C624-C632, 2001.
- Dragsten PR, Blumenthal R, and Handler JS.** Membrane asymmetry in epithelia: is the tight junction a barrier to diffusion in the plasma membrane? *Nature Lond* 294:718-722, 1981.
- Record, RD, Froelich L., Vlahos CJ and Blazer-Yost BL.** Phosphatidylinositol 3-kinase activation is required for insulin-stimulated sodium transport in A6 cells. *Am J Physiol Endocrinol Metab* 274:E611-E617, 1998.